On the Binding Modes of Metal NHC Complexes with DNA Secondary Structures: Implications for Therapy and Imaging

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Abstract

Organometallic compounds currently occupy an important place in the field of medicinal inorganic chemistry due to the unique chemical properties of metal coordination compounds. Particularly, metal compounds ligated by N-heterocyclic carbenes (NHC) have shown high potential for biomedical applications as antimicrobial and anticancer agents during the recent 15 years. Although further studies are necessary to validate the modes of action of this family of compounds, a number of biological targets have been identified, including DNA secondary structures.

This perspective review aims at providing an overview of the most representative examples of metal NHC complexes reacting with nucleic acids *via* different binding modes. It is organized according to the type of DNA secondary structure targeted by metal NHCs, highlighting the possible advantages of biomedical applications, including therapy and imaging.

1. Introduction

Since its regulatory approval for testicular and ovarian cancers in the USA in 1978, cisplatin developed into an indispensable and effective anticancer agent.[1] Its cytotoxic properties stem from direct DNA binding which induces a series of signal transduction-pathways of which some lead to cell death.[2,3,4] However, early observations of acquired drug-resistance and severe side

effects triggered the development of further derivatives of this compound's class that culminated in the approval of carboplatin and oxaliplatin.[5,6] The pharmacological activity of platinum agents was found to be affected by the ligands coordinated at the metal center and this insight was exploited as a basis for the rational design of next-generation anticancer agents.

In this context, organometallic compounds are promising candidates for future therapeutic schemes. By definition, organometallic complexes are characterized by at least one metal–carbon bond. Given that a carbon atom offers four bonding orbitals and three hybridization types, organometallic complexes yield an abundant number of stereochemically and geometrically diverse scaffolds that allow fine-tuning of their biological and physiochemical properties. Moreover, the metal–carbon bonds are kinetically inert and display high stability, essential to control metallodrugs speciation in aqueous environment. Organometallic complexes are well-established in homogenous catalysis. In particular, they are widely applied in the activation of small molecules, olefin polymerization and in different organic transformations such as carbonylation reactions and carbon-carbon bond formations.[7] Beyond that, organometallic scaffolds attract increasing interest in drug discovery because they offer a large unexplored chemical area with respect to biological and medicinal applications.[8] Typical representatives of ligands used for metal-carbon complexation include carbonyls, alkynyls, cyclopentadienyls, arenes, cyclometalated and N-heterocyclic carbenes (NHCs) as illustrated in Fig. 1.[9] As an example, Ferroquine is a ferrocene-containing antimalarial agent that currently undergoes phase II clinical trials and shows promise to overcome chloroquine resistance.[10] A cytostatic cobalt(0) complex (Co-AcS, AcS = acetylsalicylic acid) containing carbonyls and an alkyne as ligands has been shown to inhibit COX-2 *via* different binding modes than AcS alone, and the ruthenium(II) complex [Ru(η^6-arene)(PTA)Cl_2] (RAPTA-C; PTA = 1,3,5-triaza-7-phosphaadamantane) displayed strong antimetastatic activity in ovarian and colorectal

xenografts.[11,12] Consequently, it is now generally accepted that even simple modifications of organometallic scaffolds dictate target preferences.

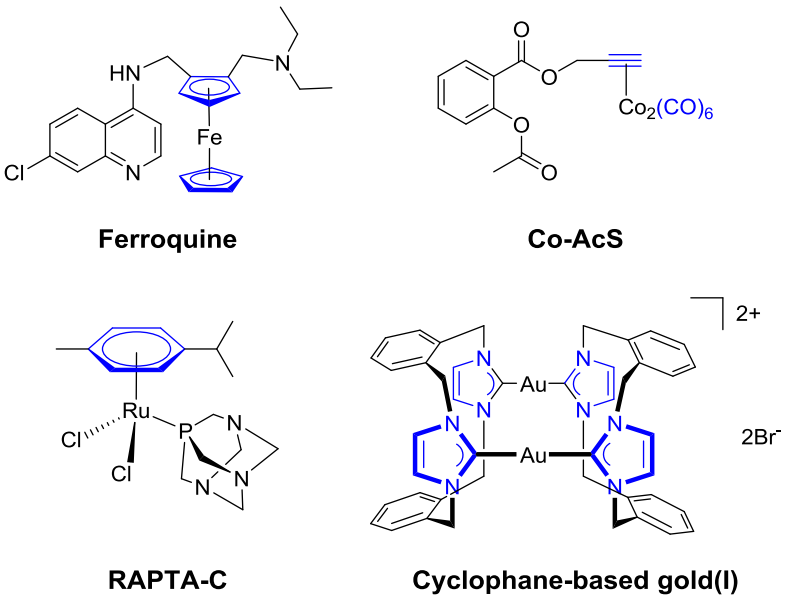


Fig. 1 - Structures of organometallic compounds used for medicinal purposes.

Within this framework, N-heterocyclic carbenes (NHCs) are of particular interest among the ligand scaffolds available for novel bioactive organometallic complexes. They consist of a neutral heterocycle with a two-electron donating carbenic carbon between two nitrogen atoms. Usually, NHCs are singlet carbenes conferring strong σ-donor properties as a ligand and low tendency to dimerize. The synthesis of the first NHC metal complex dates back to 1968, and since the first reports on free stable NHCs and their applicability as ligands in the early 1990ies[13] NHC popularity increased strongly, while even gradually supplanting the use of conventional tertiary phosphines as σ-donors. Notably, NHC carbenes form strong bonds with metals yielding organometallic complexes of high chemical and thermal stability, and they are also easily derivatised and even immobilized on carrier materials.[14]

Recent developments in inorganic drug discovery of metal NHCs, focus on coinage (Au, Ag, Cu) and platinum-group metals (Pt, Ru, Pd, Os, Ir) and their structure-activity relationships as

well as biological targets are being elucidated.[15–19,20] The biological modes of action of antiproliferative NHC complexes have been investigated for several of these metal ions and led to the identification of different potential molecular targets.[12,15–17,19–23] Among these mechanisms, cell cycle arrest and mitochondrial dysfunctions (*e.g.* ROS production, changes in mitochondrial membrane potential, cytochrome c release and caspase activation) may be responsible for triggering apoptosis in cancer cells and this is on the one hand caused by the interaction of such metal-based drug candidates with proteins.[24,25] For example, selected gold(I) NHC complexes are assumed to induce apoptosis by inhibition of thioredoxin reductase (TrxR).[26] The latter is a seleno-enzyme that is overexpressed in several cancer cells and plays an important regulatory role in the cellular redox-balance.[21,26] Other enzymatic targets of anticancer metal NHC-based complexes are glutathione reductase (GR), deubiquitinase (DUB), cyclooxygenase (COX) and cathepsin B, respectively.[24] Readers are directed to further references to cover the implication and modes of actions of metal NHCs as antimicrobial drugs.[23]

Interestingly, the cyclophane NHC-based Au(I) complex comprising an *ortho*-substituted xylylene scaffold (Figure 1) in addition to be a cytotoxic agent, is luminescent, most likely as a result of short Au(I)···Au(I) contacts (< 3 Å) induced by the cyclophane ligand framework.[27] This distance can be modulated as a function of the bridging unit within the bis-NHC ligand. Luminescence properties of metal complexes are particularly attractive for monitoring their intracellular distribution, e.g. for targeting specific cells/organs. As an example, Ag(I) and Au(I) NHC complexes bearing a fluorescent anthracenyl ligand were examined for cytotoxicity in normal and tumor cells.[28] Fluorescence microscopy experiments showed that both compounds enter cells, and are particularly efficient in penetrating tumor cells where they reach the nuclear compartment.

More recently, acridine wingtip NHC-based Au(I) complexes showed strong antiproliferative

activity and fluorescence based on IL transitions, and were found to be localized primarily in lysosomes of A549 cells by fluorescence microscopy, and in the nuclei to a lower extent.[29]

From recent studies, it emerged that NHC-derived organometallic anticancer agents show the potential to target specific DNA secondary structures and often in a purely non-covalent mode of binding. Selectivity for such DNA secondary structures is expected to increase target selectivity, while reducing off-target activity and, therefore, reducing side effects. In this perspective, we aim at categorizing DNA secondary structures as targets for organometallic NHC complexes as anticancer agents, and at highlighting the promising therapeutic gains from altering such structures. Similarly, fluorescent metal NHC probes targeting DNA secondary structures might be useful for imaging purposes to uncover their implications of in tumor biology. Thus, the different modes of binding will be discussed in the following chapters, as well as possible future developments in the field.

2. Metal NHC Complexes Targeting DNA Secondary Structures

DNA is a peculiar molecule of high complexity that carries the genomic code. Two complementary strands are associated in a helix that is condensed into chromosomes by wrapping the double helix around histone octamers as nucleosome core particles (Figure 2). However, loosening this tightly packed arrangement is required for transcription. The corresponding euchromatin can be further unfolded and forms specific structural motifs of DNA architecture. Duplex DNA, mismatched DNA and replication forks represent double-stranded motifs, while promotor G-quadruplexes (G4s), telomeric G4s and i-motifs represent intrastrand structures (Figure 2).

From a therapeutic perspective, DNA plays a prevalent role in the majority of cytotoxic therapies. For essential *in vitro* experiments DNA is easily available from natural sources

(predominantly *calf thymus* (CT)-derived). Today, it is possible to readily detect interactions of DNA with potential drugs by a variety of conclusive analytic and spectroscopic methods, involving gel electrophoresis, circular dichroism, spectroscopy, turbidimetry, DNA melting experiments as well as nuclear magnetic resonance (NMR), X-ray diffraction and mass spectrometry.[3,4,30,31] Figure 3 depicts the adducts of different DNA secondary structures with metallodrugs featuring different binding modes including (A) coordination, (B) insertion and (C) stacking, which will be discussed in this review in more detail.

Direct coordinative binding to the DNA double strand was the initial target engagement of inorganic anticancer agents, especially platinum agents. Similarly, the use of coordination-based compounds to recognize DNA in a supramolecular fashion is undisputed.[32] However, it has only more recently emerged that DNA is a structurally heterogeneous molecule that can adopt defined secondary structures, which in turn may be targeted by small molecules. Here, the aim is to provide a rationale for selectively targeting secondary DNA structures and to show that organometallic NHC complexes are especially suited as scaffolds for targeting these structures.

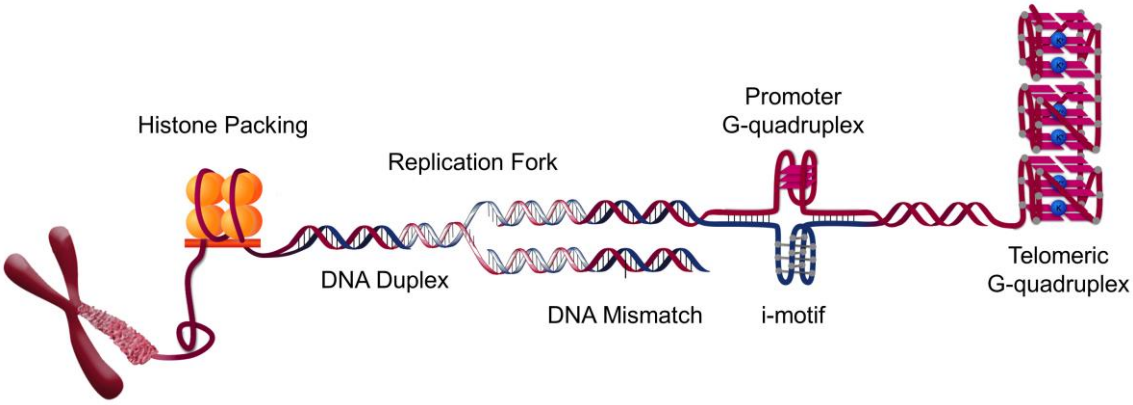


Fig. 2 - Scheme of the DNA secondary structures that may be targeted by organometallic NHC complexes.

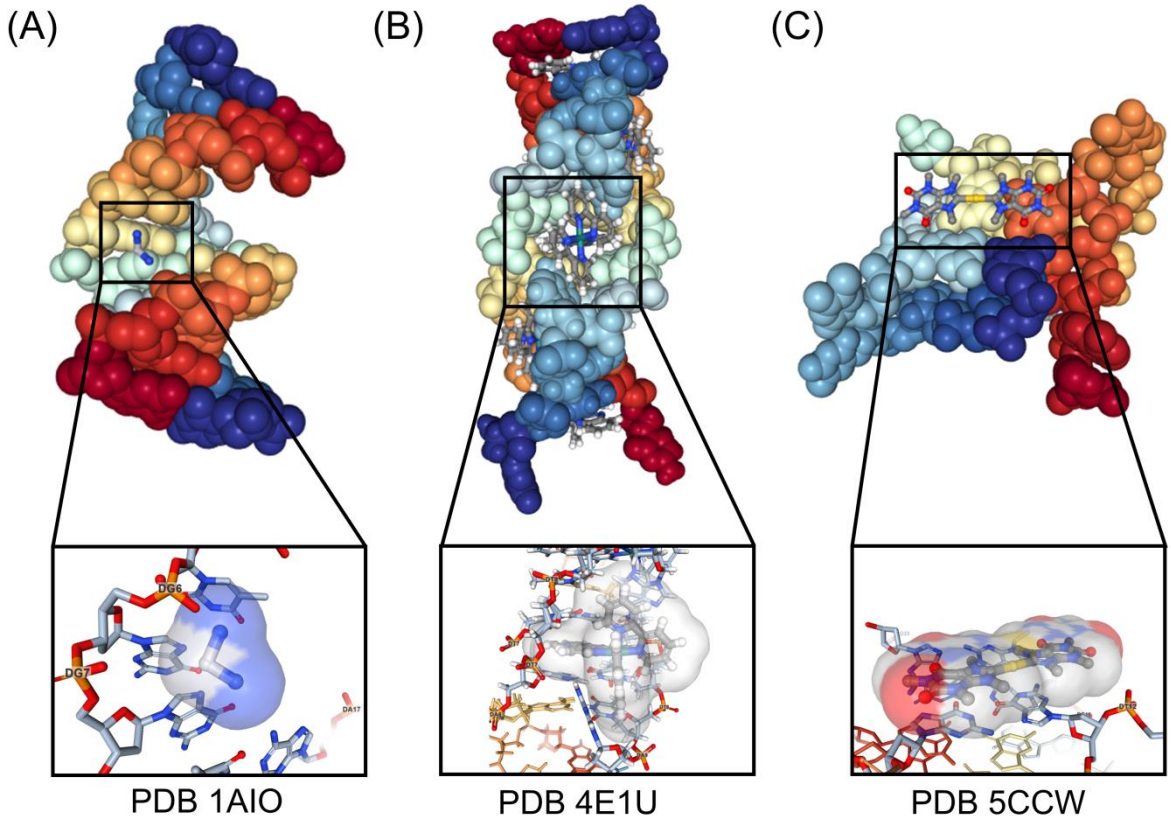


Fig. 3 - The binding modes of metal-based anticancer agents to specific DNA secondary structures are exemplified by X-ray crystal structures: (A) Activated cisplatin binding to N7 of neighbouring guanines in duplex DNA (B) Insertion of Δ-[Ru^{II}(2,2'-bipyridine)$_2$(dipyridophenazine)]$^{2+}$ into DNA mismatches A:A site (zoom) and abasic sites and (C) Binding of [Au^{I}(9-methylcaffein-8-ylidene)$_2$]$^{+}$ to a telomeric G-quadruplex *via* non-covalent interactions. The pdb accession codes are given and the structures were generated using NGL.

2.1 Targeting Duplex DNA

The double-helical architecture of DNA (duplex DNA) was one of the first therapeutic targets for cytotoxic anticancer drugs.[33] Basically, there are three biologically active double helical

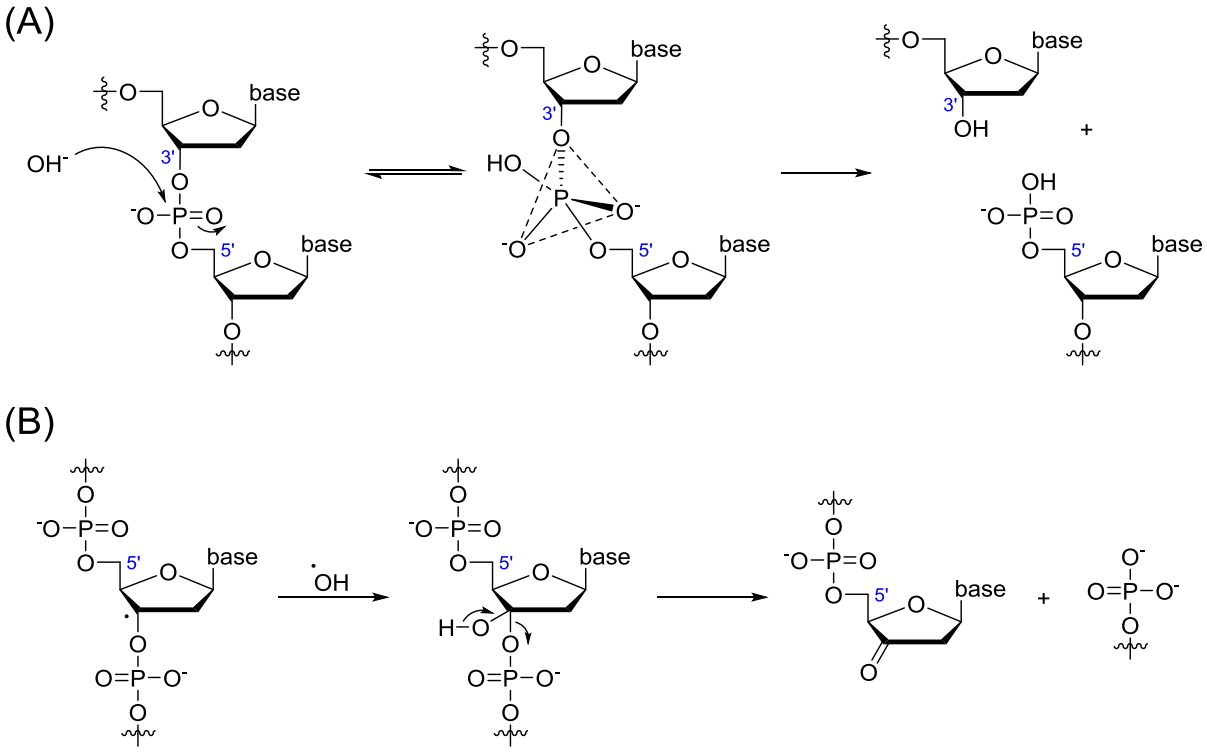
DNA structures. A- and B-DNA are made up of a right-handed helix with B-DNA being the predominate form. In contrast, Z-DNA is a left-handed helical form. The binding of metal-based drugs to DNA was mainly investigated on right-handed duplex DNA, while a few studies exist on their interaction with left-handed duplex DNA.[34] Interestingly, the reactivity of metal-based anticancer agents towards duplex DNA can be tuned to yield compounds that coordinate directly to nucleobases or intercalate/ between base pairs of duplex DNA.[32] Additionally, metal-based drug candidates can be designed to damage DNA structures by hydrolytic and/or oxidative processes. This often results in conformational changes of DNA, which disrupts transcription or DNA synthesis. Interestingly, organometallic NHC compounds based on different metal centers showed promising results in recent years as potential duplex DNA targeting anticancer agents *via* different binding modes, and representative examples are given below.

2.1.1 DNA cleavage

Double-strand cleavage of DNA is ubiquitous and essential for the maintenance of cellular functions. This process is catalysed by topoisomerases and restriction enzymes, which are nucleases and responsible for the regulation of DNA supercoiling and for defence mechanisms, respectively.[35] Interestingly, artificial nucleases were designed to interfere with DNA helicity for therapeutic purposes.[36] Nucleases cleave DNA by hydrolysis of the phosphodiester backbone of DNA. Similarly, metal-based therapeutics can undergo ligand substitution reactions with water molecules able to generate hydroxyl nucleophiles,[31,36,37] which in turn induce the nucleophilic attack on the phosphate backbone of DNA.[38] The resulting cleavage generates 5'-OPO_3 and 3'-OH fragments, which are similar to those obtained from naturally occurring nucleases (Scheme 1A).[37,39]

Moreover, certain metal complexes react with oxygen or hydrogen peroxide to form reactive oxygen species (ROS) and damage DNA in an oxidative manner[36,37] generating Fenton-type

reactions.[40] Highly reactive hydroxyl radicals (˙OH) formed in the presence of Fe(II), for example, can add to double bonds of DNA bases or abstract hydrogen atoms from the methyl group of thymine and the ribose building block, respectively.[41] Scheme 1B illustrates DNA cleavage after H-abstraction at the sugar moiety.



Scheme 1 - Postulated mechanisms of (A) hydrolytic cleavage of DNA and (B) oxidative cleavage at ribose moiety.[37]

Figure 4 shows representative cytotoxic metal NHC complexes studied for their potential activities as chemical nucleases. As a first example, Srivastava and coworkers investigated the DNA binding properties of luminescent bis-NHC complexes based on Au(I) ions (**1**).[42] Upon treatment with the gold compounds, the DNA extracted from U373-GB glioblastoma cells was

highly fragmented as monitored by gel electrophoresis assays. This effect was attributed to an elevated DNA binding affinity of the benzyl-functionalization of the carbene ligands.

A Ru(II) NHC complex (**2**) demonstrated a strong antiproliferative effect towards human colon carcinoma (HCT-15) and epidermoid cancer (Hep2) cells.[43] In gel electrophoresis assays, distinct dose-dependent DNA interactions were observed, which induced DNA cleavage, as well as DNA aggregation upon increasing the metal concentration up to 100 µM. It was suggested that **2** would intercalate into DNA and subsequently catalyze DNA cleavage.

McAlpine and coworkers showed that the incorporation of an imidazole-type NHC ligand to a rhodium(I) cyclooctadiene (COD) fragment (**3**) generated a chemical nuclease with remarkable antiproliferative activity against HCT116 colon cancer cells (IC_{50}: 3 µM).[44] Mechanistic investigations underlined DNA interactions to be causative for the antiproliferative effect. Gel electrophoresis assays revealed that this compound is capable of binding to supercoiled plasmid DNA and trigger a transformation to the nicked form. Furthermore, the restriction endonuclease BamHI was not able to digest the DNA when incubated with **3**, indicating a conformational change of the DNA upon interaction with the metallo-NHC compound. In fact, the conformational change induced by this organo-rhodium(I) compound was suggested to lead to DNA replication arrests as observed in BrdU incorporation assays with HCT116 cells, which also impaired cellular migration.

Due to their redox properties, copper compounds are well-known for their DNA cleaving activities, in particular by inducing oxidative damage.[45] Consequently, copper(I) NHC complexes bearing either one (**4**, Figure 4) or two 1,3-bis(2,4,6-trimethylphenyl)imidzolin-2-ylidene carbene ligand moieties were investigated for their interaction with duplex DNA.[46] In comparison to cisplatin, the neutral compound **4** demonstrated enhanced antiproliferative activity in several cancer cell lines. For example, the copper(I) complex exhibited an IC_{50} value of 0.075 µM in MCF-7 breast adenocarcinoma, while cisplatin has an IC_{50} of only ca. 10 µM

against the same cell line. Similarly, **4** was 150-fold more effective against HL60 promyelocytic leukemia cells compared to the platinum(II) compound. Notably, chemical nuclease activity was only demonstrated for the mono(NHC) copper(I) complex, whereas no DNA interaction is observed for the respective bis-NHC compound. Gel mobility assays demonstrated single strand cleavage of supercoiled plasmid DNA by **4** to the nicked form under aerobic conditions. Interestingly, the same experiment conducted in the presence of a singlet oxygen (NaN_3) or hydroxyl radical (DMSO) scavenger quenched this behavior. Therefore, it was concluded that **4** was DNA damaging by generating ROS only under normoxic conditions.

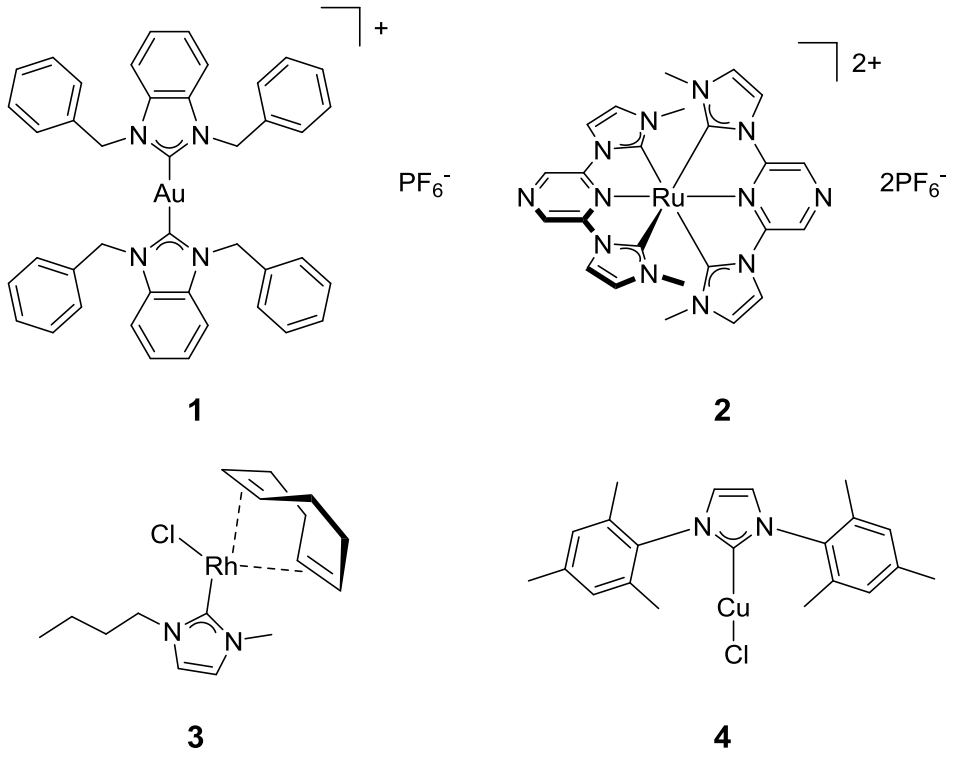


Fig. 4 - Antiproliferative metal NHC complexes studied for their potential activities as chemical nucleases.

2.1.2 Direct coordination

Alkylating agents bind irreversibly to DNA and induce conformational changes such as bending or unwinding, thus hampering vital replication and transcription processes.[3,47] The earliest examples of direct interactions between metallodrugs and DNA stem from platinum(II) anticancer compounds that coordinatively bind to DNA upon ligand exchange as revealed by X-ray crystallography (Figure 3A).[48] For example, activated cisplatin coordinates favourably to the nucleophilic N7 position of purine bases, especially guanine. Monometallic intrastrand, and to a lesser extent interstrand crosslinks, can form upon hydrolysis of both metal–halido bonds.[3,6,49]

Concerning the case of experimental gold-based anticancer compounds, their mode of action is governed by the inhibition of key proteins/enzymes and/or possible oxidative damage, leading to cell death;[21,48] while direct coordination of gold compounds to duplex DNA has been thought to be of scarce relevance. Especially those compounds bearing at least one labile ligand have been shown to be susceptible to interact with cellular nucleophiles on biomolecules, including cysteine-containing proteins (*e.g.* albumin or metallothionein) and thiol/selenol-based enzymes (*e.g.* TrxR).

Nevertheless, Gust and coworkers demonstrated that gold(I) NHC complexes **5a** and **5b** (Figure 5) – with ligands derived from pharmacologically active 4,5-diarylimidazole bearing -OCH_3 and -F substitutions – present important cytotoxic properties and moderate coordinative binding to duplex DNA.[50] In fact, measurements of the binding efficiency of the complexes to salmon testes DNA showed increased binding for **5a** and **5b** (2.03 pmol/µg and 1.03 pmol/µg, respectively) compared to Et_3PAuCl (0.11 pmol/µg), but which was still weaker than cisplatin (14.2 pmol/µg). Due to discrepancies between DNA binding studies and the respective antiproliferative activities of the complexes, the authors suggested additional mechanisms to be responsible for the biological activity.

Mono- and homobimetallic transplatin-type cytotoxic agents incorporating a NHC-ligand (**6a-b**, Figure 5) were also reported to inhibit the growth of a panel of cancer cell lines more efficiently than cisplatin.[51] This effect correlated with the strong nuclear accumulation of **6a**-**b** in A2780 and A2780-DDP cell lines, but not with the platination of DNA. For example, compound **6a** exhibited a 16-fold higher nuclear accumulation than cisplatin, but DNA platination was only enhanced 1.3-fold in A2780 cells as revealed by analyzing the treated cells with inductively coupled plasma mass spectrometry (ICP-MS). Interestingly, gel electrophoresis assays with duplex DNA, containing sequences of TGGT, TGTGT and GTTTG, respectively, demonstrated that metal-DNA adduct formation occurred and involved specific direct coordination to guanine bases.

Furthermore, novel mechanistic behaviors were reported for a polymeric formulation of platinum(II) NHC fragments attached to a bidentate polyethylenimine ligand, a known transfection agent (**7**, Figure 5).[52] Notably, the complex is much more effective than cisplatin and oxaliplatin against HCT-116 (human colorectal adenocarcinoma) cells. Evaluation of the subcellular distribution of **7** demonstrated that only 16% of the total intracellular platinum accumulated in the nucleus while 20% was found in mitochondria. Comparatively, 57% of oxaliplatin located in the nucleus whereas only 3.5% were found in mitochondria. The dual targeting of nuclei and mitochondria by **7** has been hypothesized to contribute to the promising anticancer effects in cell that exhibit resistance towards common platinum drugs. Moreover, *in vivo* experiments confirmed that **7** is more effective than oxaliplatin in reducing tumor growth.[53] In fact, an almost two-fold higher tumor volume inhibition (80%) with respect to oxaliplatin was reached in a xenograft mouse models bearing HCT-116 tumor, upon treatment with 10 mg/kg of **7** once every 48 h. Notably, no side effects were observed with compound **7** whereas the treatment of oxaliplatin induced hemorrhagic events.

Recently, Schobert and coworkers performed extensive investigations on the effect of *cis*- and *trans*-configured platinum(II) NHC complexes on biological activities including DNA interactions.[53,54] *In vitro* screening of a series of 1,3-dibenzylimidazolylidene Pt(II) complexes with different leaving groups *trans* to it (**8a-c**, Figure 5) showed that the antiproliferative activity strongly depends on the nature and position of this ligand (PPh_3, 2-picoline, Cl and NHC, respectively).[53] All complexes were effective by inhibiting the growth of different cancer cells (e.g. Panc-1, MCF-7/Topo and HCT-116) and of the cisplatin-resistant colon cancer cell line HT-29. Especially, the cationic compound **8c** exhibited IC_{50} values in the nanomolar range. Notably, the cytotoxicity followed the intuitive trend **8a** < **8b** < **8c**, according to lipophilic character. Mechanistic investigations showed that the interactions with the DNA were dependent on (*i*) the presence of a leaving group *trans* to the NHC, and (*ii*) a low bulkiness of the ligands in *cis*-position to the NHC. A different binding mode compared to cisplatin was suggested based on the minor effect of **8a**-**c** on the gel mobility of the relaxed DNA form. Interestingly, it was demonstrated that the higher the number of PPh_3, which might sterically restrict the contact with DNA or the hydrolysis of the leaving chloride, ligands, the lower the ability and rate to irreversibly bind to double-stranded CT DNA. Finally, the authors suggested that the trend of cell growth inhibition in this series was a result of the ability of the compounds to cause DNA aggregation. For example, the bis-carbene **8d** did not cause aggregation using light scattering experiments, while the mono-carbene **8c** induced the condensation of 50% of the total amount of DNA at a concentration of 0.51 μM.[54] These results, together with those of the EMSA and EtdBr studies, support the hypothesis that **8c** might interact with DNA predominantly *via* noncovalent interactions, facilitated by its positive charge.

Thereupon, *cis*-oriented bis-NHC platinum(II) complexes (**9a-b**, Figure 5) were analyzed for their anticancer activities towards different cell lines.[54] Overall, both complexes showed remarkable antiproliferative activity but the "delocalized lipophilic cationic" (DLC) **9b** was

about 10-fold more potent compared to **9a** with IC_{50} values in the nanomolar range. Electrophoretic mobility shift assays with plasmid DNA demonstrated a similar behavior of the neutral **9a** compared to cisplatin as both compounds were able to unwind the plasmid DNA in a concentration-dependent manner. Both **9a-b** induced DNA aggregation, with **9b** being the most efficient. Interestingly, analogous *trans*-bis(NHC) Pt(II) complexes did not present the same behavior.

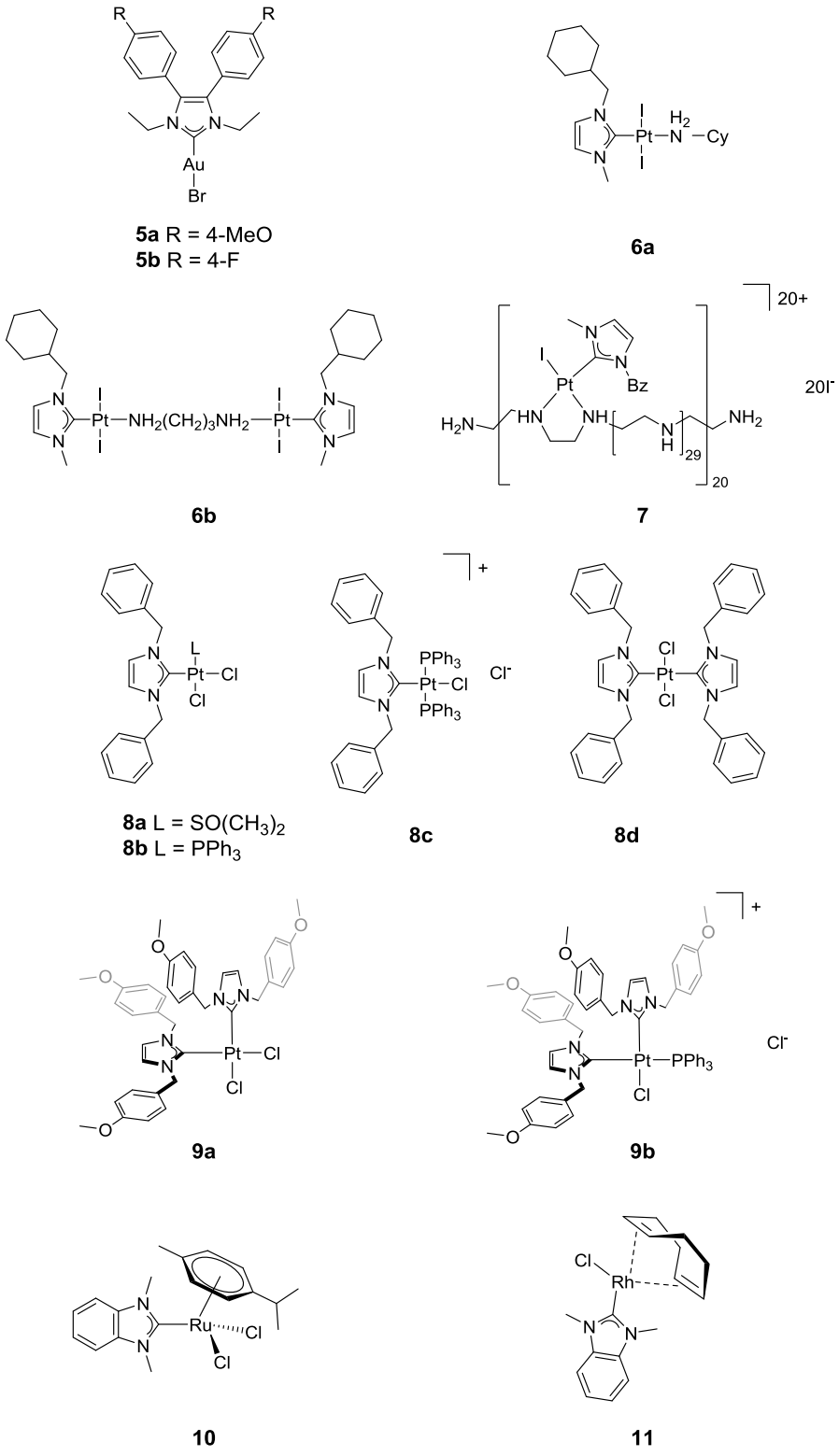


Fig. 5 - Metal NHC complexes with potential coordinative binding modes to duplex DNA.

A family of half-sandwich ruthenium(II)(arene) compounds featuring NHC ligands (*e.g.* exemplified by **10**, Figure 5) showed weak antiproliferative effects against cancer cells and ruthenation of salmon testes DNA of 15–50%, which was lower compared to cisplatin.[55] Later on, the same authors then showed the covalent binding potential of a rhodium(I) COD complex (**11**) with a similar ligand to duplex DNA.[56] In contrast to the half-sandwich ruthenium NHCs, **11** showed potent antiproliferative activity against MCF-7 human breast adenocarcinoma (IC_{50} ca. 0.6 μM) and cisplatin-resistant HT-29 colon carcinoma (IC_{50} ca. 0.9 μM) cell lines. Moreover, treatment of salmon testes DNA with **11** showed that 72% of the rhodium was bound to DNA after 4 h incubation. However, the compound metallated albumin to a similar extent (67% after 2 h). Further investigations demonstrated the potential to induce apoptosis-inducing changes of mitochondrial membrane potential in Nalm-6 leukemia cells.

2.1.3 Intercalation

Intercalation denotes the non-covalent stacking interaction of planar (heterocyclic) aromatic moieties with adjacent base pairs in duplex DNA. As a result, the double helix structure is affected and undergoes conformational changes involving lengthening, stiffening, and unwinding, respectively, which cause similar therapeutic effects compared to covalent DNA binding, *i.e.* inhibiting replication and transcription.[37] Intercalating agents such as doxorubicin and anthracyclines in general remain important and widely applied anticancer therapeutics.[32] Moreover, chloroquine completed phase III clinical trials for the treatment of glioblastoma multiform and underlines current clinical developments in this field.[57] Apart from organic small molecules, intercalating agents of various metals have been reported over the last years (*e.g.* Zn, Pt, Rh or Ru).[58] Typical metallointercalators contain cyclometalated and N-donor ligated aromatic ring systems, *e.g.* HCNN (6-phenyl-2,2'-bipyridine), phenanthroline or terpyridine, and exhibit favourably square-planar or octahedral geometries.[59]

Despite a structure that is atypical for metallointercalators, Castro and coworkers demonstrated the possibility of non-covalent DNA binding mode for the dinuclear silver mono-N-heterocyclic carbene complex **12** (Figure 6).[60] The complexes displayed antiproliferative activity against six tumor cell lines of different origin, at concentrations below 30 μM. Their interaction with CT DNA was investigated using different methods including absorption spectral titration. Thus, an increase of CT DNA amounts relative to the test compound resulted in a decrease in absorption of 20% with a slight red shift (~ 7 nm), accounting for metal complex/DNA adduct formation. Investigations on the thermal behavior of the DNA in the presence of the silver complex showed a stabilization of the double helix of CT DNA as its melting temperature increased. Consequently, the authors proposed a non-covalent reversible binding behavior of the complex to the DNA. By viscometry it was found that the silver complex induced DNA bending due to non-covalent interaction such as partial intercalation. Notably, complex **12** did not cause DNA cleavage as demonstrated by gel mobility studies.

Recently, Goite and coworkers examined DNA interactions of a dinuclear silver(I) NHC complexes with rigid pyridine linkers (**13**, Figure 6).[61] The anticancer activities of **13** were first evaluated *in vitro*, and the compound resulted moderately active. Upon titration of CT DNA, a red shift accompanied by a hypochromic effect at 237 nm (by 22%) and 280 nm (by 21%) was observed in the UV-vis absorption spectra, indicating a binding mode *via* non-covalent interaction. The viscosity of the DNA/**13** mixture is also reduced, probably as a consequence of bending or twisting of the DNA.

Similar to its corresponding silver precursor **12**, the dinuclear Au(I) NHC complex **14** was assigned to form non-covalent π-π stacking interactions with duplex DNA as a result of the modified DNA melting profiles.[60] Its cytotoxic activities against MCF-7, PC3, A549, HT-29 and 4T1 cancer cell lines were in similar ranges than for the respective silver complex, but **14** exhibited stronger interactions with DNA. Absorbance titration spectroscopy with increasing

amount of CT DNA showed clearer changes of the absorbance intensities than for compound **15**, namely a strong increase (81.8%) at 292 nm. The determined binding constant K_b ($6.32 \cdot 10^7$ M^{-1}) was also higher for Au(I) than for the Ag(I) analogue ($2.25 \cdot 10^7$ M^{-1}). A non-covalent interaction or partial intercalation was further assigned to the binding mode of Au(I) complex **14** regarding the slight decrease of the viscosity of the CT DNA/complex mixture. Interestingly, in contrast to the silver species, treatment of **14** affected the gel mobility of circular plasmid DNA (pBR322) and the authors confirmed a strong DNA interaction of the gold complex.

Likewise, the bis-gold(I) NHC complex **15** was synthesized upon transmetalation of the silver bis(carbene) intermediate **13**.[61] However, this species did not show antiproliferative effects *in vitro* up to 30 µM, which was thought to be caused by the more hydrophilic character of the gold compound (logP = -1.09 *vs.* 0.23 for **13**) most likely leading to reduced uptake. A non-covalent binding mode was observed by the viscosity reduction of the CT/DNA mixture and by the titration of increasing CT DNA equivalents with **15** that showed a strong hyperchromic effect and a decrease in intensity of only the absorbance band at 249 nm. It is worth mentioning that, in contrast to **13**, the gold(I) carbene (**15**) affected the gel mobility of plasmid DNA, as well as degraded the DNA.

A series of half-sandwich ruthenium(II) containing benzyl-substituted NHC ligands with DNA intercalating properties showed antiproliferative activities that correlated with their lipophilicity,[62] and in one case even exceeded the activity of cisplatin in prostate cancer cells (PC-3). Upon titration of the ruthenium complexes with CT DNA the resulting absorption spectra displayed a distinct hypochromism with a slight red shift, which suggested a stacking interaction between the aromatic fragments of the complexes and the DNA base pairs. Interestingly, apart from the chemical nuclease activity of the ruthenium(II) NHC (**2**), an additional intercalative binding mode was demonstrated.[12] This complex affected the morphology of CT DNA as shown by CD experiments. The negative (248 nm) as well as the

positive (272 nm) bands of the right-handed B-DNA were clearly lowered in intensity, indicating destabilization of base-stacking and loss of the right-handed helicity.

Cyclometalated ligands built from various π-conjugated organic frameworks are extensively exploited for their photoluminescent characteristics with respect to imaging applications.[63] Furthermore, the resulting planar and aromatic complex structures can promote intercalation of the metallodrug into DNA base pairs. In this context, Che and coworkers synthesized a family of cytotoxic gold(III) complexes bearing both NHC and cyclometalated C^N^C ligand moieties (*e.g.* **16**).[64] The intercalating binding mode of the complex with DNA was demonstrated by UV-Vis absorption titration and gel mobility studies. For example, upon addition of CT DNA to a solution of **16** relevant alterations of the UV-Vis spectra were observed (isosbestic points at 323 nm, 341 nm and 405 nm, and hyperchromicity of 36% at 380-420 nm) and the binding constant resulting from the intercalation was calculated as $K = 5.4 \cdot 10^5\ M^{-1}$. Further studies highlighted the potential to induce DNA strand breaks and to prevent TopoI (topoisomerase I)-mediated relaxation of supercoiled DNA. Based on the promising IC_{50} values, which were multiple times lower than for cisplatin, **16** was additionally evaluated *in vivo*. After almost a month of treatment with 10 mg/kg/week of **16**, PLC (hepatocellular carcinoma) tumor growth in nude mice models was significantly suppressed (47%) with no death or loss in body weight.

The design of metallodrugs exhibiting multiple modes of action is a promising approach in the development of novel anti-cancer drugs. In this context, an elegant method is the use of organic DNA intercalators incorporated into the ligand system of gold(I) centers which are usually associated with protein-targeting. For example, Ott and coworkers synthesized a series of gold(I) N-heterocyclic carbene compounds substituted with naphtalimides (**17a**-**d**, Figure 6), an aromatic system well-known as efficient DNA intercalator, potential cellular imaging agent and marker for cancer.[65] The intercalating properties were confirmed by circular dichroism, while the Au(I)-conjugate retained inhibitory properties to TrxR.[66] *In vitro* studies showed

antiproliferative effects against MCF-7 human breast adenocarcinoma and HT-29 colon carcinoma cells. Similar to the intercalating naphtalimide-based ligand, DNA stabilization was also observed upon treatment of CT DNA with the respective gold(I) compounds, expressed as an increase of the thermal denaturation temperature, $\Delta T_m = 11$ °C (**17a**); 6 °C (**17b**); 4 °C (**17c**); 6 °C (**17d**). Furthermore, circular dichroism (CD) spectra showed that **17a** behaved similar to the respective imidazolium salts by mainly affecting the base stacking of CT DNA. In contrast, **17b**-**d** additionally distorted the B conformation of DNA as suggested by the increase of the signal intensity at 275 nm.

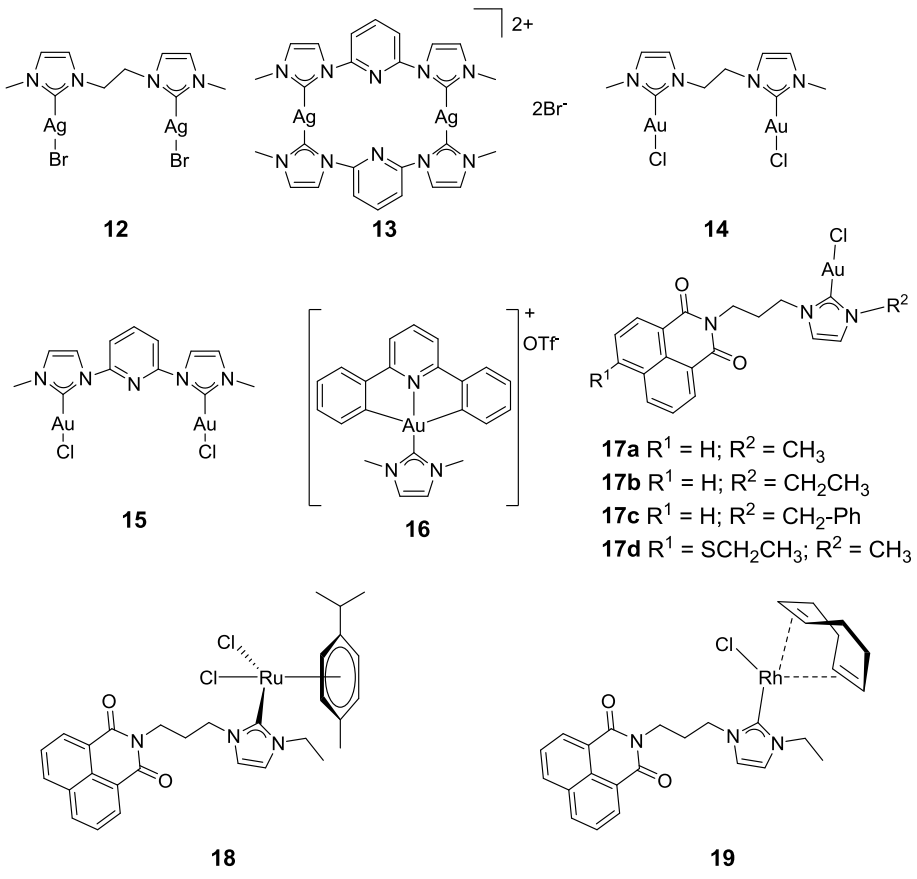


Fig. 6 - Intercalating metal NHC complexes.

Building on a similar approach,[59] NHC-based complexes exhibiting such a dual binding mode were prepared with ruthenium(II) (**18**) and rhodium(I) (**19**) ions (Figure 6).[67] Regarding the antiproliferative effects, the rhodium-based compound exhibited low micromolar activities in

the breast carcinoma (MCF-7) and colon carcinoma (HT-29) cell lines, while the ruthenium congener (**18**) was virtually inactive. Comparative mechanistic studies revealed an overall stronger DNA interaction of the rhodium-based complex **19**. UV-Vis titration absorption spectroscopy showed hypochromicity with a slight red shift indicating an intercalative binding mode. Notably, circular dichroism experiments revealed an enhanced DNA interaction of the metal-naphtalimide conjugates. Both complexes generated a band (~ 350 nm) when attached to the DNA while this effect was not observed for the free ligand. The binding constants, determined *via* UV-Vis spectroscopy and CD, displayed an overall higher DNA binding affinity of the rhodium-based **19**. Moreover, mass spectrometric studies demonstrated that both complexes were able to bind covalently to the model nucleobase 9-ethylguanine. Interestingly, photophysical properties of both compounds, deriving from the naphtalimide moiety, are quenched upon metalation.

2.2. Mismatch DNA

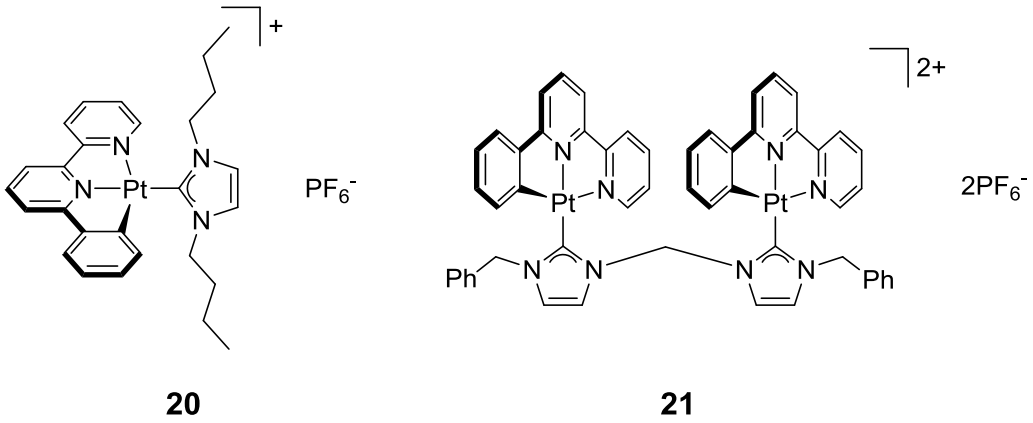
Misincorporated nucleobases during DNA synthesis that do not match the complementary base pair are termed DNA mismatches.[68] Cells evolved elaborate mechanisms, which sense and repair such defects by excision and rely on the DNA mismatch-repair machinery for this purpose. As a consequence, persistent point-mutations during DNA replication occur only at a part-per-billion frequency in healthy cells. Some colon cancers may show defects in the DNA mismatch-repair machinery and this feature may be valuable as a diagnostic or therapeutic strategy by targeting such mismatches.[69]

Some small molecules, including metal-based anticancer agents were shown to interact with mismatched DNA bases.[69,70] The first report on an intercalating ruthenium metallodrug by X-ray diffraction revealed insertion into the site of the A:A mismatch on the oligonucleotide 5'-

$(dCGGAAATTACCG)_2$-3' by expulsing both adenines, which then stacked with the ancillary metal-bound phenanthroline ligands (Figure 3B).[71] In contrast to platinum anticancer agents binding to duplex DNA *via* the major groove, this ruthenium compound inserted in the minor groove. In addition to binding to the mismatched site, the metal complex was also able to bind non-covalently to other (abasic) sites, which suggested low selectivity for the mismatch.

Recently, organometallic platinum(II) NHC complexes were reported to probe selectively mismatched DNA over matched DNA.[72] Strikingly, a $[Pt(C^N^N)(NHC)]^+$ derivative (HC^N^N = 6-phenyl-2,2'-bipyridine and NHC = n-butyl functionalized) (**20**, Figure 7), was found to selectively intercalate into C:C mismatches even over other mismatch types, *e.g.* C:T or A:C. However, this selectivity was partially reduced when extending the scaffold to a homobimetallic compound of the form $[Pt_2(C^N^N)_2(\mu\text{-}L)]^{2+}$ (L = bridging biscarbene (**21**) or diphosphine), that was expected to benefit from additional secondary stabilization of the backbone with the DNA. QM/MM calculations of a representative homobimetallic compound showed that the planar cyclometalated C^N^N ring systems inserted into the mismatch and was able to π-stack with the nucleobases, while the orthogonal backbone engages in secondary hydrophobic interactions with the expulsed nucleobases from the mismatch. Moreover, the homobimetallic species is 2+ charged, which is responsible for additional electrostatic stabilization upon binding to the DNA structure.

Based on these observations, targeting mismatched DNA by organometallic NHC compounds may prove to be a viable strategy to diagnose or treat cancer types that are characterized by a deficiency in the mismatch repair machinery. Highly selective mismatch targeting NHC compounds could be designed to feature ample secondary interactions with the expulsed nucleotides to additionally stabilize the drug–target interaction.



20 **21**

Fig. 7 - Metal NHC complexes targeting mismatched DNA.

2.3. G-quadruplexes

G-quadruplexes (G4s) are guanosine-rich intrastrand secondary structures of DNA formed by three stacks of guanosine quartets and each quartet assembles in a pseudoplanar arrangement. The hydrogen bond system is formed by a Watson-Crick edge of one guanine with a Hoogsteen edge of its neighbour, and the stacks are stabilized by two potassium ions (Figure 8A).[73] G4s play important roles in cancer progression because of their involvement in telomeric and promoter regions of the genome.[74] Several types of G4 structures have been identified so far by NMR and X-ray diffraction studies, including telomeric repeat DNA consisting of d(TTAGGG) repeats, and promoter regions of oncogenes including *MYC*, *KIT1*, *KRAS* etc. (Figure 8B).

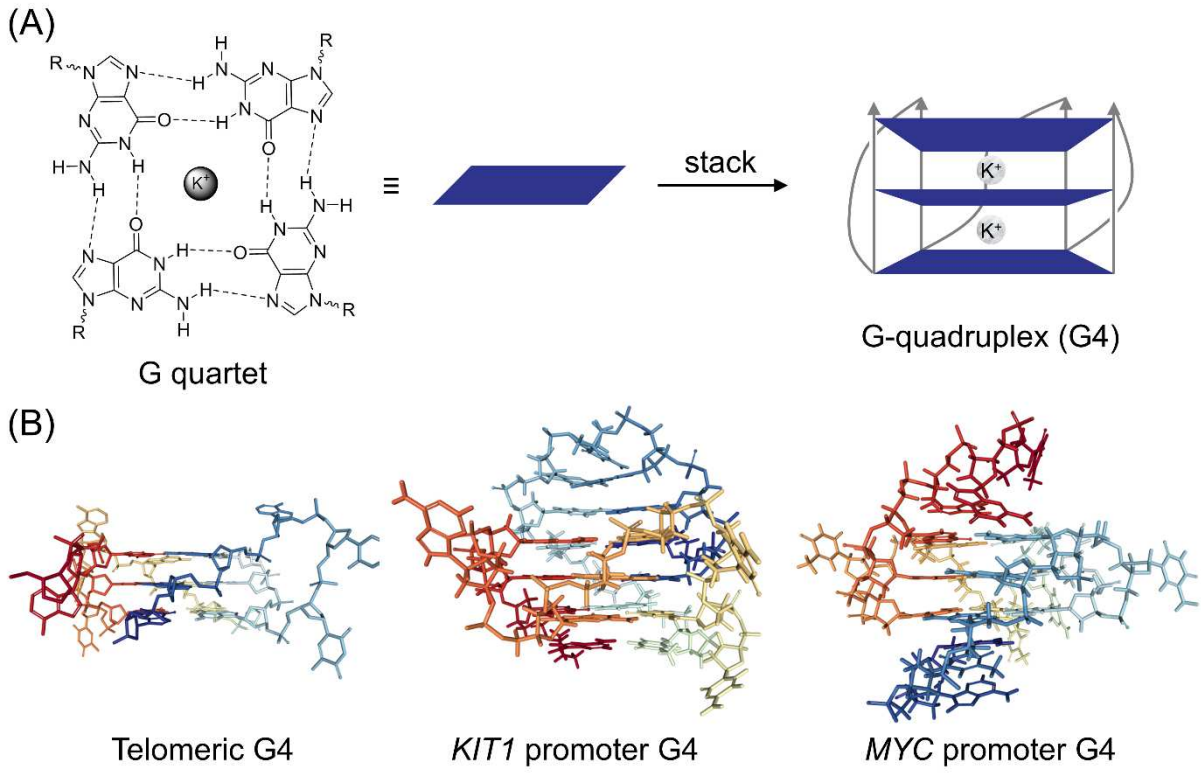


Fig. 8 - (A) Base-pairing of G-quadruplexes and (B) representative examples including telomeric G4s (*pdb* 5CCW) and promoter G4s for *MYC* (*pdb* 1XAV) and *KIT1* (*pdb* 2O3M).

2.3.1 Telomeric G4s

G4s were initially characterized as guanine-rich repeats at the single-stranded ends of chromosomes, *i.e.* the 3'-end in a single strand of about 200 nucleotides. These telomeric repeats protect against DNA degradation by folding into stacks of tightly packed G4s.[75] It was also found that the enzyme telomerase would catalyse the extension of these repeats after cellular duplication events by synthesising d(TTAGGG) telomeric repeats. Overexpression of telomerase is found in many cancer types and supports cellular immortality, which is one of the hallmarks of cancer.[76] It was therefore sought to stabilize G4s in telomeres by small molecules, which in turn would inhibit telomerase activity and thus, show anticancer effects.[75,77]

Recently, a bis-NHC gold(I) complex - $[Au(9\text{-methylcaffein-8-ylidene})_2]^+$ (**22**, Figure 9) - bearing the natural product caffeine as carbene ligand, has been demonstrated by DNA FRET melting assays to selectively targeting telomeric G4 structures with respect to duplex DNA, while showing selective antiproliferative effects on tumorigenic over healthy cells.[78] The G4 consisted of a 23 nucleotide telomere repeat sequence d(TAGGG(TTAGGG)$_3$. The X-ray structure indicated that the loops of the telomeric G4 do not cover the G-quartets, but extend to the side of the structure in a propeller configuration (Figure 8B). This feature allows telomeric G4s to form stacks and the planar **22** was then shown to bind non-covalently between neighboring telomeric G4s.[79] Moreover, the 3,3´-end accommodated one and the 5,5´-end accommodated two $[Au(9\text{-methylcaffein-8-ylidene})_2]^+$ ions. This compound shows characteristic features for G4 stabilization including *(i)* a planar structure *(ii)* delocalized guanine analogues suitable for π-stacking and *(iii)* a central positively-charged gold(I) atom adding electrostatic interactions.

Preliminary structure-activity relationships on this compound family revealed that N9-derivatizations of the xanthine backbone affected the selectivity over duplex DNA. This is exemplified by the methylbenzoate derivative $[Au(9\text{-}\{4\text{-methylbenzoate}\}\text{caffein-8-ylidene})_2]^+$ (**23**, Figure 9). The bulky ligand brakes the planarity of the organometallic compound and results in an orthogonal arrangement of the xanthine ligands.[78] This derivative was equally potent, but less selective for telomeric G4 over duplex DNA compared to the methyl-derivative and suggests that it may interact with telomeric G4 in a different manner. Compound **22** and analogues were evaluated for their toxicity in an *ex vivo* model that uses tissue slices.[78] Notably, the xanthine-derived compounds were not toxic in healthy tissues, at variance with the benzimidazole-derived analogues.

An additional example of an organometallic NHC-type stabilizer of telomeric G4s was reported by conjugating a *trans* platinum(II)-NHC moiety to pyridostatin (**24**, Figure 9).[80] The latter is

a selective telomeric G4 binder and was expected to direct the platinum(II)-type moiety to specific locations in the nucleus, where the Pt(II) moiety could then bind form to G4s *via* irreversible coordinative bonds. The compound was shown to cause distinctly different cellular effects compared to cisplatin and displaced the telomere maintenance protein TRF2 from telomeres, but not to such an extent as to induce telomere shortening. Although conclusive structural information has not yet been provided for the interaction of **24** with DNA at a molecular level, the displacement of TRF2 provides evidence that **24** targets telomeric DNA in cellular systems, and represents one of the few examples of a validated target engagement in cells.

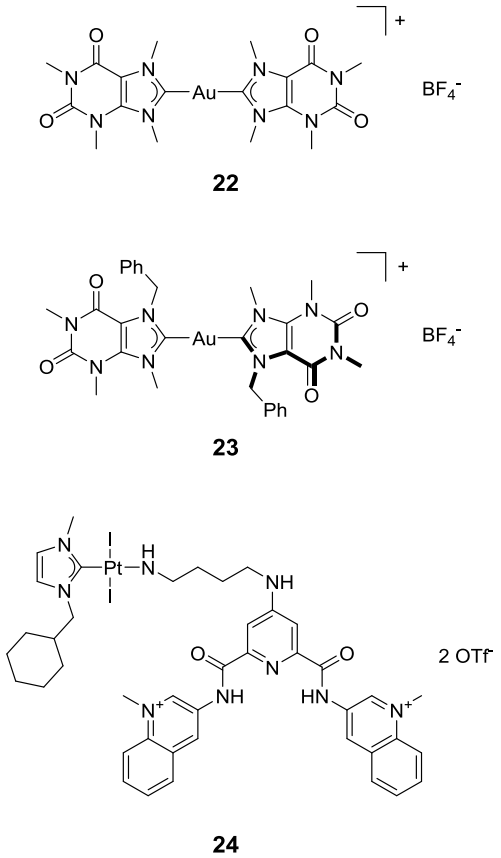


Fig. 9 - NHC metal complexes targeting G-quadruplex DNA.

2.3.2. Promoter G4s

In addition to telomeric G4s, it was shown using genome sequencing that approximately 700,000 DNA G4-like sequences,[81] while bioinformatic studies reported around 350,000 sequences with the potential to form G4 structures.[82] Finally, immunoprecipitating G4 structures from cells together with high-throughput sequencing revealed approximately 10,000 G4 structures in human chromatin.[83] It was revealed that these G4 sequences were especially present in non-nucleosomal regions in the genome and that they would enrich in promoter regions of genes. Promoter G4s occur once per promoter region and although small in size, they show specific architectures according to the arrangement of the loops connecting the guanine stacks, which in principle allow for specific drug targeting, *e.g.* the G4 sequence in the promoter region of the KIT1 gene is largely conserved and unique in the human genome, and so is presumably its structure. KIT is a proto-oncogene encoding a receptor tyrosine kinase that stimulates proliferation.[84] It has been proposed that formation of the quadruplex structure in these regions may control transcription and as a consequence, the expression of the corresponding oncogenes. Thus, stabilizing G4s by small molecules may be utilized to treat KRAS, KIT1 or c-MYC driven cancers, which are inherently difficult to treat, *e.g.* lung adenocarcinoma.[85,86] Promoter G4s are intrastrand structures on duplex DNA and the complementary strand displays a C-rich sequence that may arrange into the so-called i-motif, which is one of the few known cases of systematic base intercalations.[87] This complementary structure is less investigated than the G4 but may prove to be equally useful in a therapeutic or diagnostic context.

3. Conclusions and Future Directions

The use of metal carbene complexes in various fields beyond catalysis has experienced substantial advances during the last years, including for biomedical applications. Recent proof-of-principle studies on organometallic NHC anticancer agents showed that this class of compounds is able to target several canonical and non-canonical DNA secondary structures. In detail, metal NHC complexes were shown to interact with duplex, mismatched and G-quadruplex DNA, while examples of other classes of metal-based anticancer agents exist that inhibit replication forks,[89] by interacting with DNA three-way junctions,[90] which in the future may become amenable to targeting by NHC ligated organometallics. Concerning the type of interaction, metal NHC complexes feature different DNA binding modes, including coordinative and intercalating, and some of them show induction of oxidative damage; thus, revealing the potential of organometallic scaffolds to allow a plethora of strategies to target nucleic acids, for either therapy or imaging. For example, $[Au(NHC)_2]^+$ complexes with appropriately functionalized wingtip groups constitute components that combine luminescent and antiproliferative properties in the same scaffold. Such chemical tools would be ideal to study the predominate questions of metallodrugs in cells with regard to metal speciation and validation of the pharmacological mechanisms. In fact, a combination of fluorescence spectroscopy and isotope-specific mass spectrometry would allow to follow not only the compounds distribution in cells, but also the structural integrity of the compound, *e.g.* with regard to ligand exchange reactions. This can even be extended by X-ray absorption near-edge spectroscopy (XANES) to study the composition of the first coordination sphere and the oxidation state of metallodrugs in tissues.[91] Notably, advanced mass spectrometric techniques emerge as promising tools to generate hypotheses on target identification[92] and mechanisms of action in the cellular context.[93]

Furthermore, recent developments in the DNA targeted field of organometallic NHC anticancer agents show also a trend towards molecular and systems biology approaches in order to

elucidate mechanisms of action or effective signal transduction pathways and *in situ* chemical affinity capture will presumably increase in popularity to validate binding of selected compounds to specific DNA secondary structures *in vitro*.[86] Similarly, ELISA microarrays may prove useful to globally elucidate signalling events upon drug administration.[94]

It is evident that future research in the discovery and development of organometallic NHC compounds has to be directed towards a more rational drug discovery of therapeutic compounds with respect to the targeting of specific DNA secondary structures. Such molecules may be developed also as chemical probes to aid in uncovering the functions of these structures in cancer biology in more detail.

Although the spatial arrangement of the nucleotides in DNA secondary structures may be unique, it remains to be proven whether specific DNA secondary structures can be targeted at all given the complexity of genomic DNA. In particular, targeting of G4s of specific genes would enable a powerful approach for gene silencing at the level of transcription initiation and would be equivalent to a small molecule gene knock-down. Of note, although telomeric and promoter G4s show marked structural differences, it remains challenging to develop selective agents for either type, although coordination metal-based compounds have shown already interesting properties.[95] Similarly, current research efforts are directed towards small molecules that target specific promoter G4s. Moreover, despite intensive research in this area, the actual function of G4s *in vivo* has not yet been fully understood. Therefore, significant efforts have been devoted to the discovery of specific probes for visualizing and distinguishing G4 structures from other nucleic acid molecules likely to be found in biological environments.[96] In this context, based on the representative examples described in this review, metal NHCs certainly hold great promise.

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